Molecular Identification and Epidemiological Tracing of *Pasteurella multocida* Meningitis in a Baby

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We report a case of *Pasteurella multocida* meningitis in a 1-month-old baby exposed to close contact with two dogs and a cat but without any known history of injury by these animals. 16S rRNA gene sequencing of the isolate from the baby allowed identification at the subspecies level and pointed to the cat as a possible source of infection. Molecular typing of *Pasteurella* isolates from the animals, from the baby, and from unrelated animals clearly confirmed that the cat harbored the same *P. multocida* subsp. *septica* strain on its tonsils as the one isolated from the cerebrospinal fluid of the baby. This case stresses the necessity of informing susceptible hosts at risk of contracting zoonotic agents about some basic hygiene rules when keeping pets. In addition, this study illustrates the usefulness of molecular methods for identification and epidemiological tracing of *Pasteurella* isolates.

A previously healthy 1-month-old baby from a rural area of Switzerland was admitted to the pediatric ward of the local hospital in the winter of 1998 to 1999. The baby presented with an irritable state, a temperature of 39°C, and signs of slightly increased intracranial pressure. A lumbar puncture was performed, and the cerebrospinal fluid (CSF) was positive for numerous polymorphonuclear leukocytes and small gram-negative bacilli. After 24 h of incubation, a small, catalase-positive, oxidase-positive and indole-positive gram-negative coccobacillus grew on sheep blood and chocolate agar plates, as well as in thioglycolate enrichment broth. Formal identification of *Pasteurella multocida* was achieved using the API 20NE system (bioMérieux, Marcy-l’Etoile, France). The organism was susceptible to all tested beta-lactam antibiotics. The patient was successfully treated with ceftriaxone (400 mg/day) for 2 weeks, until complete recovery. An inquiry revealed that the baby had no brother or sister but had been in close contact with two dogs and possibly a cat through a misguided attempt by the parents to promote bonding between the baby and the family’s pets. This suggested a probable animal source of infection.

Shortly after the identification of the incriminated pathogen, swab samples were taken from the throats and tonsils of the parents, of the two dogs, and of the cat which had been in contact with the baby. Swabs from the parents were cultured on 5% sheep blood agar, on chocolate agar, and on MacConkey agar plates. Phenotypic identification of *Pasteurella multocida* was achieved using the API 20NE system (bioMérieux, Marcy-l’Etoile, France). The organism was susceptible to all tested beta-lactam antibiotics. The patient was successfully treated with ceftriaxone (400 mg/day) for 2 weeks, until complete recovery. An inquiry revealed that the baby had no brother or sister but had been in close contact with two dogs and possibly a cat through a misguided attempt by the parents to promote bonding between the baby and the family’s pets. This suggested a probable animal source of infection.

No *Pasteurella* organisms could be detected and isolated from the cultures made with the samples taken from the parents. However, a *Pasteurella* strain was isolated from the tonsils of the cat which had been in contact with the baby. This isolate had the same *P. multocida* biochemical profile as the isolate from the CSF of the baby (typical profile for *P. multocida*, trehalose-negative and sorbitol-positive reactions). 16S rRNA gene sequencing and comparison with our reference database led to the unambiguous identification of these two isolates as *P. multocida* subsp. *septica* (100% identity with the *P. multocida* subsp. *septica* type strain, 0.28% divergence from the less closely related *P. multocida* subsp. *septica* isolate of our database, and 1.85% divergence from the most closely related isolate of the two other *P. multocida* subspecies). A second isolate from the cat (oral cavity) showed a biochemical profile compatible with an unidentifiable *Pasteurella* species. This lat-
From these cases, followed by a smaller proportion of the major sources of infection for humans (11). *P. multocida* is a common cause of infections in pets, an often forgotten source of zoonoses. In the particular case, the isolates from one another, except those from the baby and the cat had identical ribotypes with both restriction enzymes *Hind*III and *EcoRI* following the manufacturer’s instructions (Roche Diagnostics, Rotkreuz, Switzerland). The resulting fragments were separated by agarose gel electrophoresis and transferred by vacuum blotting on positively charged nylon membranes (Roche Diagnostics). A digoxigenin-labeled probe specific for 16S rRNA gene was prepared by PCR and used for hybridizations, and the hybridization patterns were revealed using the digoxigenin luminescence kit (Roche Diagnostics). For macrorestriction analysis, bacteria were grown on sheep blood agar plates and resuspended in TE (10 mM Tris, 1 mM EDTA, pH 8.0) to a final optical density at 600 nm of 4.0. The suspensions were mixed with an equal volume of melted 1.2% SeaKem Gold agarose (FMC, Rockland, Maine), and 1-mm-thick plugs were prepared. The plugs were incubated overnight at 50°C in 0.5 M EDTA–1% lauryl sarcosine containing 2 mg of proteinase K per ml and washed five times for 45 min each time in TE. The DNA was subsequently digested for 4 h with 30 U of *SalI* or *SmaI* under conditions described by the manufacturer (Roche Diagnostics). The resulting DNA fragments were separated with a CHEF III electrophoresis system (Bio-Rad, Hercules, Calif.) in 1% SeaKem Gold agarose gels (FMC) for 16 h in 0.5 × Tris-borate-EDTA at 14°C by applying an electric field of 6 V/cm with an angle of 120°C and a linear ramping ranging of 1.5 to 17 s. Gels were stained in 1 mg of ethidium bromide per ml and destained in water before photography under UV light. Results of ribotyping and macrorestriction analysis are summarized in Table 1. Only three very similar ribotypes could be distinguished when using the restriction enzyme *Hind*III, with nine isolates belonging to a single type (Table 1). Three of these ribotypes were clearly different from those recently described for *P. multocida* subsp. *multocida* (9), thus confirming their identification as *P. multocida* subsp. *septica*. *EcoRI* was more discriminatory (Table 1), but the profiles obtained with this enzyme remained relatively similar and were difficult to read because of the presence of variable bands in the high-molecular-weight range (data not shown). The isolates from the baby and from the cat had identical ribotypes with both restriction enzymes. Macrogen restriction profiles were easy to read and allowed us to distinguish all of the *P. multocida* subsp. *septica* isolates from one another, except those from the baby and the contact cat, which remained identical (Fig. 1).

Cattle, pigs, and poultry have been incriminated in the past as the major sources of human infections with zoonotic agents. However, humans have very frequent and close contacts with pets, an often forgotten source of zoonoses. In the particular case of *Pasteurella* infections, animal bites and scratches are the major sources of infection for humans (11). *P. multocida* subsp. *multocida* represents the majority of *Pasteurella* isolates from these cases, followed by a smaller proportion of *P. multocida* subsp. *septica* isolate from the cat. Our knowledge, this is the first report of a *P. multocida* isolate from a contact.}
FIG. 1. Macrorestriction analysis of *P. multocida* subsp. *septica* isolates. (A) Macrorestriction profiles of *Pasteurella* isolates obtained after digestion with SfiI. Lanes: 1 to 9, *P. multocida* subsp. *septica* isolates from unrelated cats; 10 and 11, *P. multocida* subsp. *septica* isolates from the baby and from the contact cat, respectively. (B) Macrorestriction profiles of *Pasteurella* isolates obtained after digestion with Smal. Isolates are in the same order as in panel A. The DNA in lane 1, isolate remained uncut because of methylation.

subsp. *septica* meningeal meningitis in which the organism was transmitted without any injury from a cat to a baby to be clearly documented by genotypic and molecular epidemiological methods. The baby had been massively exposed to the dogs by scratching on the head. In this study, the present work exemplifies the need for molecular identification methods for taxa such as members of the family *Pasteurellaceae*. Finally, our results also clearly illustrate the potentials of pulsed-field gel electrophoresis for epidemiological tracing of the occurrence of *P. multocida* infections in humans.

REFERENCES